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Optimizing the use of potassium sorbate and sodium metabisulphite for the chemical and microbial stability of carbonated coconut water

Otimizando o uso de sorbato de potássio e metabissufito de sódio para a estabilidade química e microbiológica de água de coco carbonatada

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Summary

Coconut water is popular worldwide, mainly because of its pleasant sensory characteristics, nutritional value and low calorie density. However, coconut water is a highly perishable product due to the presence of enzymes such as peroxidase and polyphenoloxidase, which cause undesirable changes in colour, and also because of its susceptibility to microbial spoilage. The use of chemical additives has been adopted by the industry with the intent of increasing product shelf life. In this study, the efficiency of the preservatives potassium sorbate and sodium metabisulphite was assessed using a Central Composite Rotational Design (CCRD) to determine the stability of carbonated coconut water, varying the concentrations of potassium sorbate from 0 to 500 mg.L^{-1}, and of sodium metabisulphite from 0 to 100 mg.L^{-1}. The chemical evaluations included carbonation volume, pH, soluble solids, dissolved oxygen and carbon dioxide, acidity, ascorbic acid, polyphenoloxidase and peroxidase activities, colour and turbidity attributes. The microbiological evaluations considered the total aerobic plate count and the enumeration of yeasts and moulds. It was observed that concentrations of 375 mg.L^{-1} of potassium sorbate and 75 mg.L^{-1} of sodium metabisulphite gave the best quality attributes with respect to minor changes in acidity and colour of the coconut water, providing that the raw material had low microbiological contamination.

Key words: *Coconut water; Carbonation; Potassium sorbate; Sodium metabisulphite; Microbiological quality.*

Resumo

A água de coco é muito popular em todo o mundo, principalmente em virtude das suas características sensoriais agradáveis, do valor nutricional e das baixas calorias. No entanto, a água de coco é um produto altamente perecível em função da presença de enzimas – tais como peroxidase e polifenoloxidase, que causam alterações indesejáveis na cor – e também em função da sua susceptibilidade à deterioração microbiana. O uso de aditivos químicos tem sido adotado pela indústria com a intenção de aumentar a vida de prateleira do produto. Neste estudo, a eficiência dos conservantes sorbato de potássio e metabissulfito de sódio foi avaliada considerando-se a estabilidade da água de coco carbonatada por meio do delineamento composto central rotacional (DCCR), variando as concentrações de sorbato de potássio entre 0 e 500 mg.L^{-1}, e de metabissulfito de sódio entre 0 e 100 mg.L^{-1}. Foram realizadas avaliações físico-químicas de volume de carbonatação, pH, sólidos solúveis totais, oxigênio e dióxido de carbono dissolvidos, acidez, ácido ascórbico, atividade de polifenoloxidase e peroxidase, cor e atributos de turbidez. As análises microbiológicas realizadas foram de contagem total de aeróbios, bem como de enumeração de bolores e leveduras. Observou-se que as concentrações de 375 mg.L^{-1} de sorbato de potássio e 75 mg.L^{-1} de metabissulfito de sódio apresentaram os melhores atributos de qualidade, no que diz respeito a pequenas alterações na acidez e à cor da água de coco, desde que a matéria-prima apresentasse contaminação microbiológica baixa.

Palavras-chave: *Água de coco; Carbonatação; Sorbato de potássio; Metabissulfito de sódio; Qualidade microbiológica.*

1 Introduction

Coconut water is a beverage appreciated worldwide. It is a drink with a slight sweet and acid flavour (pH 5.5), it is somewhat cloudy and it is constituted mainly of minerals and sugars, and in smaller proportions, by nitrogenous substances (amino-acids), lipids and vitamins. Due to its rich composition in salts, it is considered as a natural isotonic drink (MEDINA et al., 1980; MACIEL et al., 1992; CAMPOS et al., 1996).

Coconut water is a highly nutritious beverage which favours microbial spoilage. The product is also susceptible to browning due to high oxido-reductase enzyme concentrations such as polyphenoloxidase and peroxidase.

Polyphenoloxidase catalyzes two types of oxidative reactions: hydroxylation of monophenols into o-diphenols and the oxidation of the latter compounds, which are colourless, into dark toned o-quinones (DUARTE et al., 2002).

Peroxidase, on the other hand, catalyzes a great number of oxidative reactions using hydrogen peroxide as the substrate, or, in some cases, oxygen as a hydrogen acceptor. Peroxidase is considered to be the most heat stable vegetable enzyme and its inactivation has conventionally been used as an indicator of blanching efficacy in vegetable processing (FREITAS et al., 2008).

Enzymatic browning is usually undesirable and therefore chemical and physical methods have been developed aiming at its inhibition, which can be achieved by the elimination or complexation of one or more essential components of the reaction such as oxygen, enzyme, copper or substrate (GUERRERO-BELTRÁN et al., 2005).

The only normative instruction in Brazil concerning processed coconut water applies to its physicochemical characteristics (BRASIL, 2009), and there is no specific legislation concerning the use of preservatives for this product. Thus different compounds are used according to the specific legislation for fruit juices, that is, 0.004 g.100 mL^{-1} of sodium sulphite and 0.03 g.100 mL^{-1} of sorbic acid in the carbonated beverages (BRASIL, 2007). Ascorbic acid is frequently used to prevent enzymatic browning, and has been shown to be more effective than its isomer, iso-ascorbic acid. This acid acts as an antioxidant, reducing the quinine produced by polyphenoloxidase, and contributing to a drop in the pH value. However, ascorbic acid can also participate in the non-enzymatic browning process by forming furfural derivatives in the presence of low pH and heating, producing dark pigments (QUEIROZ et al., 2008; TORALLES et al., 2008).

In a study by Abreu and Faria (2007), ascorbic acid was applied at concentrations of 0, 100 and 200 $mg.L^{-1}$, together with heat treatments varying between 138 and 144 °C for 10 seconds. They found that a heat treatment of 139 °C for 10 seconds plus the addition of 200 $mg.L^{-1}$ of ascorbic acid provided the best physicochemical stability for aseptically packed coconut water.

Sulphites are widely used to prevent browning, either by enzymatic or non-enzymatic means. They are also used in the control of microorganisms as antioxidants or reducing agents, amongst other functions. However, due to its adverse health effects, the World Health Organization (WHO) limits the use of sulphur dioxide (SO_2) in processed food products to a maximum daily dose of 0.7 $mg.kg^{-1}$ of body mass (QUEIROZ et al., 2008; FAO, 1967).

Although sulphur dioxide and sulphites are generally recognized as safe (GRAS) substances, their use in wines is limited to 0.035 % because higher levels could lead to undesirable flavours. The use of sulphites is not allowed in foods that are considered as sources of thiamine since they inactivate this vitamin (MAGA and TU, 1994; MITCHELL, 1990).

According to Maga and Tu (1994), the intake of sulphite at normal levels in foods does not result in accumulation in the body, because it is rapidly oxidized into sulphate and excreted in the urine. However, a dose above tolerable levels (such as 62 $mg.kg^{-1}$ of body mass) of sulphur dioxide has resulted in neurological problems in rats, including polyneuritis, visceral atrophy, bone marrow atrophy, renal dysfunction and growth limitation. In direct contact with the eyes, SO_2 is quickly absorbed and gets into the cornea, causing acute inflammation. However, in general, no mutagenic, teratogenic or carcinogenic effects of SO_2 have been observed in rats or mice (MAGA and TU, 1994; MITCHELL, 1990). However, sulphites have been associated with asthmatic attacks and other allergenic effects, and in extreme cases they are able to cause death due to hypersensitivity.

Sorbic acid and sorbates are generally used in the control of microorganisms in industrialized food products, with a maximum recommended daily intake of 12.5 $mg.kg^{-1}$ of body mass (MITCHELL, 1990; FAO, 1967). For the microbiological preservation of coconut water, many authors have used pre–determined concentrations of preservatives such as: 40 $mg.L^{-1}$ of sodium metabisulphite (CARVALHO et al., 2007a, b); 500 and 600 $mg.kg^{-1}$ of potassium metabisulphite (CHOWDHURY et al., 2005); 45 $mg.L^{-1}$ of sodium metabisulphite and 124 $mg.L^{-1}$ of sodium benzoate (SILVA et al., 2003), despite the fact that no study has been carried out to determine the minimum concentration necessary to preserve coconut water.

The present research analyzed the combined action of potassium sorbate at concentrations ranging from 0 to 500 $mg.L^{-1}$ with sodium metabisulphite at concentrations from 0 to 100 $mg.L^{-1}$. These concentrations were chosen in order to obtain samples containing only one of the preservatives and also concentrations above those

permitted by the Brazilian law. The effect of carbonation was also assessed, since it is generally known to provide a refreshing sensation and also because of a possible role in microbial growth inhibition.

2 Experimental procedures

2.1 Materials

Green coconuts of the dwarf variety were used with maturity levels of approximately 6 to 7 months, bought from the Central Supply Unit in Campinas – SP (CEASA), Brazil. For standardization of the coconut water (Brix = 7 °Brix and pH between 4.3-4.5), refined sugar was used as a source of sucrose (Caravelas brand, Usina Colombo, Ariranha – SP, Brazil), and citric acid (Synth brand, Labsynth, Diadema – SP, Brazil). Ascorbic acid (Nuclear brand, Casa da Química, Diadema – SP, Brazil) was used at a concentration of 200 mg.L^{-1}, and the chemical preservatives potassium sorbate (Vetec brand, Vetec, Rio de Janeiro – RJ, Brazil) (varying from 0 to 500 mg.L^{-1}) and sodium metabisulphite (Synth brand, Labsynth, Diadema – SP, Brazil), (varying from 0 to 100 mg.L^{-1}) were also employed.

As for the packaging system, transparent polyethylene terephthalate (PET) bottles were used, provided by Minalba (Minalba, São José dos Campos - SP). The characteristics of the bottles were: weight of 16.49 ± 0.05 g, volumetric capacity of 348.58 ± 1.32 mL and oxygen permeability of 0.047 ± 0.001 cm^3/bottle. day.atm at 25 °C. High density polyethylene screw caps containing Bericap seal liners (Sorocaba-SP) were used to close the bottles,.

3 Methods

Before extracting the water from the fruits, they were sanitized using a solution of sodium hypochlorite (200 mg.L^{-1}). The water was then standardized to a pH between 4.3-4.5 and 7 °Brix using citric acid and sucrose. At this point, 200 mg.L^{-1} of ascorbic acid were added to the water. The drink was clarified by passing through a 1 µm pore opening filter, pasteurized at 90 °C for 30 seconds, and rapidly cooled to 2 °C in a plate heat exchanger (model Micro Plak Jr., manufactured by Suma Brand Indústria e Comércio Ltda., Campinas – SP, Brazil), with a nominal flow rate of 300 L.h^{-1}, fed with a positive displacement pump (Netzsch brand, Pomerode – SC, Brazil). The coconut water was carbonated to 2 to 3 volumes in a carbonating machine developed by Faria (2007). This step consisted of injecting carbon dioxide into the beverage under pressure to obtain the desired degree of carbonation. The drink was packaged into PET bottles, previously cleaned with a sodium hypochlorite solution (200 mg.L^{-1}) and stored at room temperature (20 ± 1 °C) for 62 days. The preservatives were added before carbonation. The concentration used was established by a Central Composite Rotational Design (CCRD) with two independent variables (potassium sorbate and sodium metabisulphite) at concentrations varying from 0 to 500 mg.L^{-1} and 0 to 100 mg.L^{-1}, respectively, as shown in Table 1. The responses obtained were statistically analyzed using the STATISTICA 7 program.

3.1 Evaluation of stability

To evaluate the stability, the carbonation volume was analysed according to the ASTM F1115-95 standards (ASTM, 2001); the pH was determined using a Digimed potentiometer (Sao Paulo – SP, Brazil), model DM-20 at 25 °C; the soluble solids were determined using a portable refractometer (Optech, model RCZ, Guarulhos – SP, Brazil); dissolved O_2 was assessed using an O_2 meter (Mettler Toledo, model MO128, Barueri – SP, Brazil); dissolved CO_2 was evaluated using a CO_2 electrode (Thermo Scientific, model Orion 720A - represented in Brazil by Analyser, Sao Paulo - SP); colour and turbidity were determined in a colorimeter (Hunterlab, model Colorquest II, using the CIELAB system with the illuminant D65, observer's angle of 10°, TTRAN-type calibration and HAZE measurement - turbidity).

Titratable acidity was determined based on method 942.15 of AOAC (HORWITZ, 1997). The ascorbic acid concentration was determined by titrating 10 mL of sample with 50 mL of a 1 % oxalic acid solution standardized with a solution of 2 g.L^{-1} dichloroindophenol (DCFI). A spectrophotometer (Beckman, model DU-70) was used to determine the peroxidase and polyphenoloxidase activities. For polyphenoloxidase, 1.3 mL of 0.35M phosphate buffer (pH 6.0), 0.7 mL of 0.2M catechol and 2 mL of the coconut water sample were mixed at room temperature, and the absorbance read at 425 nm in a spectrophotometer at zero time and after 10 minutes of reaction. For peroxidase, 1.3 mL of 0.35M phosphate buffer (pH 5.5) at 35 °C was used, and 2 mL of the coconut water sample added together with 0.5 mL of an alcoholic solution of 0.5 % guaiacol and 0.2 mL of 0.1 % hydrogen peroxide . The absorbance was read at 470 nm

Table 1. Design Matrix for 2^2.

Assay	A	B	C	D	E	F	G	H	I	J	K
Potassium Sorbate (mg.L^{-1})	73	427	73	427	0	500	250	250	250	250	250
Sodium Metabisulphite (mg.L^{-1})	15	15	85	85	50	50	0	100	50	50	50

in a spectrophotometer at zero time and after 10 minutes of reaction.

For the microbiological evaluations, the total aerobic plate count was obtained by pour plating 1 mL of each dilution into Plate Count Agar (PCA). The colonies were counted after 48 hours of incubation at 35 °C and expressed as Colony Forming Units per mL (CFU.mL^{-1}). The yeast and mould count was obtained by surface plating 1 mL of each dilution in Potato Dextrose Agar (PDA) and counting after 5 days at 23 °C (SILVA et al., 2010). The microbiological evaluation was carried out at zero time and after 30 and 61 days of storage.

4 Results and discussion

The samples were evaluated during 62 days of storage at room temperature (20 ± 2 °C). At the end of the storage period, it was shown that the concentration of the preservatives influenced the results with a significance of 10 % for the carbonation volume, pH, dissolved CO_2, titratable acidity, ascorbic acid concentration, colour b*and turbidity attributes, as shown in Table 2.

Ignoring the non-significant effects, the following 7 equations were obtained, and it was also possible to calculate the analysis of variance (ANOVA) for each of the attributes, as presented in Table 3.

$$\text{Carbonation volume} = 2.07 - 0.24x_1 - 0.18x_2 + 0.15x_2^2 \quad (1)$$

$$pH = 4.05 + 0.06x_1 + 0.03x_2 - 0.02x_2^2 \quad (2)$$

$$CO_2\left(mg \cdot L^{-1}\right) = 4960.45 - 158.16x_2 \quad (3)$$

$$\text{Titratable acidity } (\text{mL NaOH 0.1N / 100mL of sample}) = 36.88 - 3.86x_1 + 2.28x_1^2 \quad (4)$$

$$\text{Ascorbic acid}\left(mg \cdot L^{-1}\right) = 173.68 + 16.82x_1 - 19.13x_1^2 \quad (5)$$

$$\text{Colour b*} = 5.98 - 1.30x_1 + 0.99x_1^2 - 3.13x_2 \quad (6)$$

$$\text{Turbidity} = 43.17 - 12.20x_2 \quad (7)$$

where: x_1: Concentration of potassium sorbate and x_2: concentration of sodium metabisulphite.

All regressions were significant with a significance level of 10 %. It was possible to construct response surfaces for each attribute as presented in Figure 1.

The response surface for carbonation volume (Figure 1a) showed an inverse correlation, with lower concentrations of the two preservatives giving greater carbonation volumes. However, the inverse behaviour was observed for pH (Figure 1b), with lower concentrations of the two preservatives giving lower pH values.

Table 2. Estimated effects for a second order fit, representing the relationship between the responses and process variables.

	Estimated effects											
	Carbonation	pH	CO_2	SS	Acidity	AA	PER	PFO	L* Colour	a* Colour	b* Colour	Turbidity
Average	2.00*	4.05*	4813.33*	6.80*	35.87*	175.75*	0.0233	0.0267	93.70*	-0.47*	4.89*	33.88*
(1) x1 (L)	-0.48*	0.12*	-149.36	0.38	-7.72*	33.64*	0.0775	0.1580	0.59	-0.19	-2.59*	-12.95
X1 (Q)	0.15	-0.01	89.79	0.22	5.18*	-39.54*	0.0154	0.1121	-0.65	-0.07	2.66*	7.59
(2) x2 (L)	-0.37*	0.05*	-316.31*	1.13	-2.07	8.79	-0.0775	-0.2469	0.40	0.16	-6.26*	-24.40*
x2 (Q)	0.35*	-0.05*	314.79	-0.85	2.13	-4.39	0.0154	0.0946	0.34	0.21	2.31	17.97
(1) x (2)	0.37	-0.03	-327.50	-0.20	-1.27	17.58	-0.1550	-0.2700	0.67	0.40	0.18	13.89

*Significant estimated effects at 10 %. CO_2: concentration of dissolved CO_2 in mg.L^{-1}; SS: soluble solids in °Brix; Acidity: measured in mL of NaOH 0,1N/100mL; AA: concentration of ascorbic acid in mg.L^{-1}; PER: peroxidase activity in U.mL^{-1}; PFO: polyphenoloxidase activity in U.mL^{-1}.

Table 3. ANOVA.

	Carbonation		pH		CO_2		Acidity		AA		b* Colour		Turbidity	
	LD	SQ	LD	SQ	LD	SQ	LD	SQ	LD	SQ	LD	SQ	LD	SQ
Regression	3	0.8692	3	0.0396	1	200105.26	2	151.11	2	4524.88	3	97.79	1	1190.57
Residue	7	0.3565	7	0.0047	9	522317.47	8	36.23	8	1315.77	7	18.43	9	1617.71
Lack of fit	5	0.2356	5	0.0047	7	461050.81	6	33.99	6	1315.77	5	17.59	7	1614.97
Pure Error	2	0.1209	2	0.0001	2	61266.67	2	2.24	2	0.00	2	0.84	2	2.75
Total	10	1.2257	10	0.0444	10	722422.73	10	187.34	10	5840.65	10	116.22	10	2808.28
R^2	70.92		89.34		27.7		80.66		77.47		84.14		42.39	

LD: liberty degrees; SQ: sum of the squares; CO_2: concentration of CO_2 dissolved in mg.L^{-1}; Acidity: measured in mL of NaOH 0,1N/100mL; AA: concentration of ascorbic acid in mg.L^{-1}.

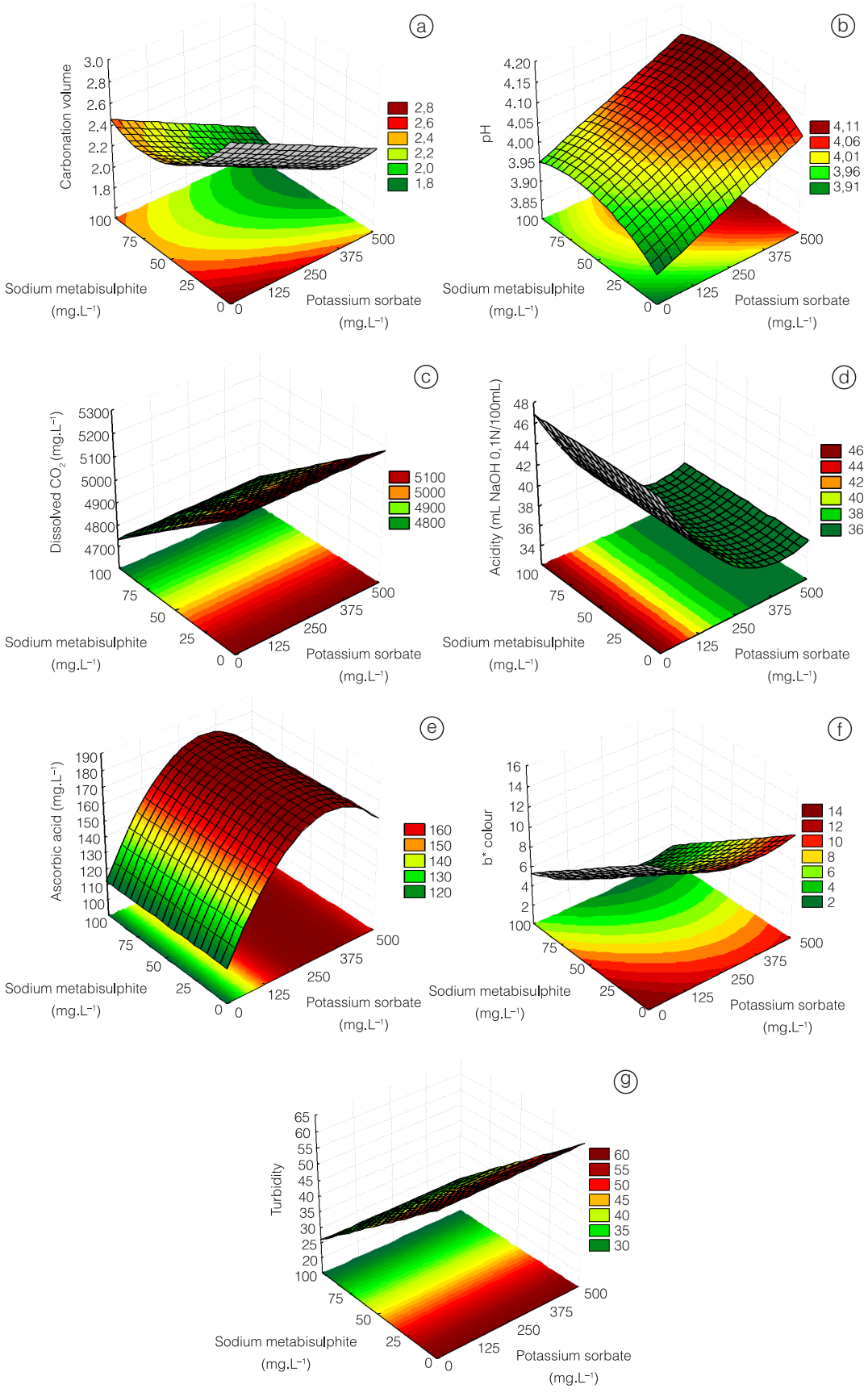

Figure 1. Response surfaces for: (a) carbonation volume, (b) pH, (c) dissolved CO_2, (d) titratable acidity (e) ascorbic acid concentration, (f) b* colour and (g) turbidity.

In the determination of dissolved CO_2, (Figure 1c), only sodium metabisulphite showed a significant response, with lower concentrations of this preservative giving rise to greater quantities of dissolved gas. As for acidity (Figure 1d) only potassium sorbate was significant, and the lower its concentration, the higher the acidity developed in the sample.

In the determination of ascorbic acid, only potassium sorbate showed a significant response, and the lower the concentration of potassium sorbate, the greater the degradation of ascorbic acid, as seen in Figure 1e. Lima et al. (2009) also found a decrease in ascorbic acid concentration in coconut water-acerola fruit juice supplemented with caffeine, at a rate of 1.24 mg.100 mL^{-1}.day^{-1}.

For the b* colour attribute, low sodium metabisulphite concentrations, and very low or very high potassium sorbate concentrations favoured the development of a yellow colour (higher values for b*) (Figure 1f). However for turbidity, only sodium metabisulphite had a significant effect, with lower concentrations allowing for the development of more turbidity in the samples (Figure 1g). These results could be associated with the growth of microorganisms forming gas and acid, since the sample would be less protected due to the lower concentrations of preservative, as shown in Table 4.

The microbiological evaluations of the samples (formulations presented in Table 1) were carried out at zero time and after 30 and 61 days of storage at room temperature, according to Table 4. Samples A, B, C, E and G presented a total microbial count above 10^3 CFU.mL^{-1} after 30 days of storage. Samples A, B and C showed decreases in the microbial counts after 61 days, which could be attributed to the start of the decline phase of microbiological development. Samples E and G showed an increase in the microbial count at the end of the observation period. With the exception of sample B, all the samples contained the lowest concentrations of the preservatives, and samples E and G only contained sodium metabisulphite and potassium sorbate, respectively. These results indicated there was a certain synergism between the two preservatives with respect to microbial control. The other samples, D, F, H and K, showed no significant microbial development at any point.

For the yeast and mould counts, samples A, B, C, E, G and I presented detectable counts after 30 days of storage, with E and G having counts above 10^5 CFU.mL^{-1}. After 61 days, there was an increase in the yeast and mould counts for samples A, E and G, with E and G showing total counts above 10^6 CFU.mL^{-1}, a fact that confirms the efficacy of the combined action provided by the two preservatives.

The activities of the enzymes polyphenoloxidase and peroxidase were not significant in any of the samples during the storage time, indicating that the thermal processing applied to the samples was sufficient to inactivate them. As in the study of Murasaki-Aliberti et al. (2004), heat processing with shorter holding times can minimize the nutritional and sensory losses in coconut water and promote 90 % enzymatic inactivation.

Based on the present results, concentrations of 375 and 75 mg.L^{-1} of potassium sorbate and sodium metabisulphite, respectively, caused less changes in all the attributes evaluated. However, such concentrations are not allowed by the Brazilian legislation (BRASIL, 2007), with limits of 260 mg.L^{-1} for potassium sorbate and 40 mg.L^{-1} for sodium metabisulphite. According to the present results, this combination would also be sufficient to preserve the product, and thus additional confirmatory trials were carried out using these concentrations, as well as the optimal combination. However, these confirmatory trials showed very high microbiological contamination, with counts of over 10^8 CFU.mL^{-1} over a period of 20 days of storage for both samples, indicating that these concentrations of the preservatives were not sufficient to

Table 4. Total and yeast and mould counts for samples A, B, C, D, E, F, G, H, I, J and K, after 0, 30 and 61 days of storage.

	Sample	A	B	C	D	E	F	G	H	I	J	K
0 days	Total Count (CFU.mL^{-1})	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	Yeasts and Moulds (CFU.mL^{-1})	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$
30 days	Total Count (CFU.mL^{-1})	$1.6 . 10^3$	$2.3 . 10^3$	$5.0 . 10^3$	<1.0	$2.9 . 10^3$	<1.0	$5.0 . 10^3$	<1.0	<1.0	$5.0 . 10^0$	<1.0
	Yeasts and Moulds (CFU.mL^{-1})	$2.8 . 10^4$	$1.0 . 10^3$	$5.0 . 10^3$	<1.0	$1.9 . 10^5$	<1.0	$1.4 . 10^5$	<1.0	$5.0 . 10^2$	<1.0	<1.0
61 days	Total Count (CFU.mL^{-1})	$5.0 . 10^1$	$5.0 . 10^1$	<1.0	<1.0	$5.8 . 10^4$	<1.0	$5.9 . 10^4$	<1.0	<1.0	<1.0	<1.0
	Yeasts and Moulds (CFU.mL^{-1})	$9.2 . 10^5$	<1.0	<1.0	<1.0	$1.4 . 10^6$	$1.0 . 10^0$	$1.1 . 10^6$	<1.0	<1.0	$5.0 . 10^1$	<1.0

maintain the microbiological quality of the product through the storage period when the raw material had a high microbiological load. In these confirmatory trials, the initial counts were over 10^2 CFU.mL^{-1} for the total count and almost 10^1 CFU.mL^{-1} for the yeasts and moulds. This last result highlights the importance of good manufacturing practices in order to reduce or avoid contamination of the product.

5 Conclusion

The present results indicated that the samples presented less alterations when higher concentrations of the preservatives were added. However, such concentrations were above the limits established by Brazilian law. For the sample with concentrations of 260 mg.L^{-1} of potassium sorbate and 40 mg.L^{-1} of sodium metabisulphite, which are the limits established by the legislation, good stability of the carbonated coconut water was observed for approximately 60 days of storage at room temperature, provided the initial microbial counts were low. It was also observed that the samples that received only one type of preservative presented less stability, demonstrating a possible synergistic effect between potassium sorbate and sodium metabisulphite. Moreover, this study showed that the addition of CO_2 to the coconut water, besides giving a more refreshing sensation to the beverage, also contributed to its conservation because it helped to reduce the pH value of the product, as well as reducing the amount of dissolved oxygen.

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